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ARTICLE

Predictive value of cystatin C and beta-2 microglobulin in preeclampsia

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Abstract The purpose of this study was to determine whether the levels of cystatin C and beta-2 microglobulin (B2M) are altered during the second trimester in the plasma of women who subsequently develop preeclampsia.

Study design: We performed a case control study to compare the levels of cystatin C and B2M in women in whom preeclampsia ultimately developed ($n = 30$) and in pregnant women who remained normotensive throughout gestation ($n = 60$). The maternal plasma levels of cystatin C and B2M were measured by enzyme-linked immunosorbent assay. Blood samples were collected between 15 and 20 weeks' gestation for fetal aneuploidy screening and frozen at −20 °C until assay after groups had been selected.

Results: The median concentrations of cystatin C and B2M were significantly higher in those who subsequently developed preeclampsia when compared to those of normal pregnancy (median 668.6 ng/ml and 418.3 μg/ml vs 413.7 ng/ml and 321.2 μg/ml, respectively).

Conclusions: In this study, the maternal plasma levels of cystatin C and B2M were significantly elevated in pregnant women who subsequently developed preeclampsia as compared with

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normotensive women. Alterations of these proteins antedate clinical symptoms and, thus, they may be useful for early identification of patients at the risk of developing preeclampsia.

1. Introduction

Preeclampsia (PE) is a multisystem disorder that is specific to human pregnancy. It is defined as the association of pregnancy-induced hypertension with proteinuria that occurs after 20 weeks of gestation [31]. It is associated with high maternal mortality and morbidity as well as the risk of perinatal death, preterm birth, and intrauterine growth restriction [24]. It has been reported to complicate 4–7% of pregnancies worldwide [17].

Although the cause of preeclampsia remains elusive, the origin of the condition is recognized as lying in the placenta. This is because preeclampsia occurs only in the presence of pregnancy and it resolves after delivery of the placenta [2].

Studies during the past decade have provided a better understanding of the potential mechanisms responsible for the pathogenesis of PE. The initiating event in PE appears to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles [6].

It has been hypothesized that placental ischemia is an early event, leading to the placental production of a soluble factor or factors that cause maternal endothelial dysfunction resulting in the clinical findings of hypertension and proteinuria. Proteinuria and hypertension dominate the clinical picture, because the chief target organ is the kidney (glomerular endotheliosis) [11]. Thus, an altered renal function is an essential component of the pathophysiology of the disorder. Implantation of the placenta and vascular changes are completed by 20–22 weeks of gestation. So although PE is usually diagnosed in the second half of pregnancy, damage has already occurred at an earlier stage of pregnancy [3].

β2 Microglobulin also known as B2M is a component of MHC class I molecules [19]. Possessing an Ig-like domain, B2M is non-covalently associated with a heavy chain of HLA antigens [18].

Cystatin C is a nonglycosylated protein that belongs to the cysteine protease inhibitors, cystatin superfamily. It is found in virtually all tissues and body fluids. It is a potent inhibitor of lysosomal proteinases (enzymes from a special subunit of the cell that break down proteins) and probably one of the most important extracellular inhibitors of cysteine proteases (it prevents the breakdown of proteins outside the cell by a specific type of protein degrading [26,5]).

B2 microglobulin and cystatin C are freely filtered by the glomerulus and reabsorbed and catabolized by proximal tubular cells. Therefore, their serum values could be a better marker of glomerular filtration rate (GFR) than serum creatinine level [15]. Cystatin C values will be abnormally high when GFR decreases to 88–95 ml/min per 1.73 m^2 [14,13]. Some studies found B2M to be less adequate than cystatin C as a GFR marker [7,4], but another did not show any differences [14].

A previous study carried out by Kristensen and colleagues [16] has reported that the plasma levels of cystatin C and beta-2-microglobulin are significantly elevated in preeclampsia and that these low molecular mass proteins are useful markers of renal impairment in preeclampsia. High levels of cystatin c in PE might reflect placental ischemia [22].

The aim of this study is to determine whether a change in the levels of cystatin C and beta-2-microglobulin could be detected before the onset of PE, and thus, these two markers could be used to predict the subsequent development of preeclampsia.

2. Subjects and methods

This is a case-control study in which each woman in whom preeclampsia ultimately developed was matched with 2 women who remained normotensive throughout gestation. All women were free of pre-existing hypertension, diabetes, renal disease and gave birth to singletons. Written informed consent was obtained from the women agreeing to participate in the study.

Maternal serum samples were originally collected between 15 and 20 weeks of gestation for the purposes of antenatal screening for fetal aneuploidy and neural tube defects. This screening program is available to all pregnant women attending the Prenatal Diagnosis Clinic, at the National Research Center. Serum samples were frozen at −20 °C until assay after groups have been selected.

The study group comprised 90 pregnant women. The first group (Group 1) consisted of 30 pregnant women who developed preeclampsia. The second group (Group 2) consisted of 60 women as controls, who were normotensive throughout pregnancy and delivered infants who were appropriately sized.

Preeclampsia was defined as either a systolic blood pressure over 140 mm Hg or a diastolic blood pressure over 90 mm Hg on two occasions, at least 6 h apart, occurring after 20 weeks of gestation. Proteinuria was defined as either >300 mg of protein in a 24-h urine collection or two positive dipstick results of at least 1+ (30 mg/dl) on two occasions 4-h apart, or urine dipstick results of at least 2+ (100 mg/dl) with no evidence of urinary tract infection. Blood pressure was measured after a 10 min rest, with the subject in the lying position.

All serum samples were measured in duplicates and the mean of the duplicates was used for the statistical analysis. Cystatin C concentration was measured by a quantitative ELISA technique using Quantikine human cystatin C immunoassay (R&D Systems Europe Ltd.). The intra-assay and inter-assay coefficients of variation (CV) were less than 6.6 and 7, respectively. Beta-2-microglobulin was measured using ORG 5 BM immunoassay (Orgentec Diagnostica GmbH). The intra-assay and inter-assay CV were less than 3.6 and 4.9, respectively.

2.1. Statistical analysis

The data were processed and analyzed using the program (SPSS) statistical package for social sciences version II under windows XP. Descriptive statistics were performed for

Table 1 Clinical characteristic of the study population.

	Preeclampsia $N = 30$	Controls $N = 60$	P value
Maternal age (years)	28.8 ± 5.26	28.4 ± 4.77	NS
Gestation at delivery (weeks)	38.1 ± 0.88	39.8 ± 0.79	< 0.001
Diastolic BP (mm Hg)	97.0 ± 4.22	71.5 ± 7.84	< 0.0001
Systolic BP (mm Hg)	143.0 ± 5.87	112.0 ± 7.523	< 0.0001

P value ⩽0.0001 is highly significant.

Table 2 Median levels of cystatin C and B2M in cases and controls.

	Preeclampsia $N = 30$	Controls $N = 60$	P value
Cystatin C (ng/ml)	668.6 (71.7–1427.7)	413.7 (348.3–1177.1)	< 0.0001
B2M (μg/ml)	418.3 (150.4–570.0)	321.2 (98.5–548.1)	< 0.0001

P value ⩽0.0001 is highly significant.

categorical data using percents for quantitative data using the mean and standard deviation. Comparison of the means was done using the paired "t" test with statistical significance level preset at 0.05 level [23].

3. Results

This study was conducted on 90 pregnant women attending the Prenatal Diagnosis Clinic, at the National Research Center, Cairo. All women were matched for age, gestational age at sampling (15–20 weeks) and freezing time of samples. They were divided into two groups according to the outcome:

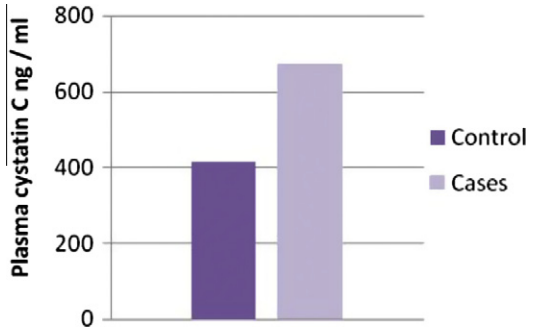

Figure 1 Plasma cystatin C (ng/ml) levels in cases and control.

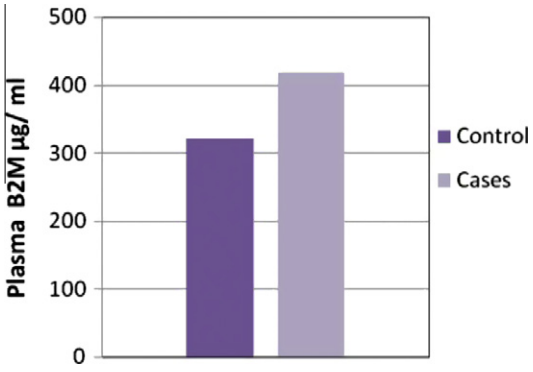

Figure 2 Plasma B2M (μg/ml) levels in cases and control.

Group I: Thirty pregnant women who later developed preeclampsia after 20 weeks.
Group II: Sixty pregnant women who remained normotensive throughout the pregnancy.

The clinical characteristics of each group are presented in Table 1. There were no significant differences between the two groups in terms of age or parity, but there was a significant difference in the gestation at delivery as women who developed PE delivered early compared to normal pregnant women.

The plasma concentrations of cystatin C and B2M were significantly higher in women who developed preeclampsia compared to normotensive women (668.6 ng/ml vs 413.7 ng/ml, $P \leqslant 0.0001$; 418.3 μg/ml vs 321.2 μg/ml, $P \leqslant 0.0001$, respectively). Table 2 shows values of cystatin C and B2M in samples taken before 20 weeks. Fig. 1 shows plasma cystatin C ng/ml levels in cases and control. Fig. 2 shows plasma B2M μg/ml levels in cases and control.

4. Discussion

In this study we found significantly the elevated levels of cystatin C and B2M in the second trimester in the plasma of women who subsequently developed preeclampsia compared to normal pregnant women. We chose 15–20 weeks as the gestation for screening, because this is emerging as the visit at which the biochemical testing for chromosomal and other defects is carried out. Cystatin C and B2M are low molecular weight proteins produced by all nucleated cells at a constant rate. They are freely filtered by the glomerulus and reabsorbed and catabolized by proximal tubular cells [25,21]. Cystatin C is a cysteine protease inhibitor with a molecular mass of 13 kDa [7]. Serum cystatin C concentration is reported to be unaffected by muscle mass, age, inflammation, fever, or exogenous agents [29]. However, there are reports that the thyroid function has an impact on cystatin C concentrations [30]. The protein B2M is an 11.7 kDa nonglycosylated polypeptide composed of 99 amino acids. Increased plasma levels of B2M occur in a variety of autoimmune illnesses, infectious or inflammatory process, and proliferative syndromes [14,10].

Although preeclampsia appears to begin in the placenta, the target organ is the maternal endothelium [12]. Preeclampsia is considered the most common glomerular disease in the world [27]. Its etiology and pathogenesis remain poorly understood. It is characterized by the new-onset hypertension and proteinuria, in association with a characteristic glomerular lesion "endotheliosis". Glomerular endotheliosis is a characteristic lesion found in renal biopsies of preeclamptic women, it represents a specific variant of thrombotic microangiopathy and has been considered as the hallmark of PE [9].

Hypertension has been attributed to an excess of vasoconstrictor over vasodilator influences in the systemic circulation. A parallel imbalance in the renal circulation would be expected to lower the glomerular perfusion rate. Limited glomerular ultrafiltration capacity caused by endotheliosis in combination with glomerular underperfusion, results in depression of the glomerular filtration rate (GFR) [27].

A study concerning serum levels of B2M in PE and normal pregnancy was published by Noyan et al. [20]. These investigators have found serum B2M concentrations to be significantly elevated in preeclamptic patients compared with normal pregnant women, while no difference was found in B2M levels in normotensive pregnancy compared with non-pregnant women.

Our data regarding both cystatin C and B2M levels are consistent with the previous studies of Thilaganathan et al. [28] and Saleh et al. [22]. Kristensen et al. [16] found the elevated plasma concentrations of cystatin C and B2M in patients with preeclampsia at the time of diagnosis, and, both were displaying a similar diagnostic performance for diagnosing preeclampsia. Although other studies found that B2M concentrations were not predictive of the development of preeclampsia [8,1].

Accurate prediction of PE is important for identifying those women who require more intensive monitoring, permitting earlier recognition and intervention to those at risk, thus improving the maternal and fetal outcomes.

Thus, the ability to predict early in pregnancy those women at high risk for PE might lead to decreased maternal and fetal morbidity and mortality.

5. Conclusion

Our results showed that cystatin C and B2M levels were increased in the second trimester plasma of women who subsequently developed preeclampsia compared to normal pregnant women. Although cystatin C and B2M may be potential markers for predicting preeclampsia, further large scale studies are essential to determine their usefulness.

Limitations of the study are the small sample size. Furthermore, the population of the study was entirely Egyptian, thus, further work is needed to determine whether levels of these biochemical markers differ among different ethnic groups.

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